Neurosci. Bull. February, 2017, 33(1):53–61
DOI 10.1007/s12264-016-0073-2

www.neurosci.cn
www.springer.com/12264

ORIGINAL ARTICLE

Expression of SoxC Transcription Factors during Zebrafish Retinal and Optic Nerve Regeneration

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Received: 18 April 2016 / Accepted: 23 July 2016 / Published online: 14 October 2016

Abstract The SoxC transcription factors (Sox4, Sox11, and Sox12) play important roles in the development of the vertebrate eye and retina. However, their expression and function during retinal and optic nerve regeneration remain elusive. In this study, we investigated the expression and possible functions of the SoxC genes after retinal and optic nerve injury in adult zebrafish. We found that among the five SoxC members, Sox11b was strongly induced in BrdU-positive cells in the inner nuclear layer (INL) after retinal injury, and morpholino-mediated Sox11b-knockdown significantly reduced the number of proliferating cells in the INL at 4 days post-injury. After optic nerve lesion, both Sox11a and Sox11b were strongly expressed in retinal ganglion cells (RGCs), and knockdown of both Sox11a and Sox11b inhibited RGC axon regrowth in retinal explants. Our study thus uncovered a novel expression pattern of SoxC family genes after retinal and optic nerve injury, and suggests that they have important functions during retinal and optic nerve regeneration.

Keywords SoxC · Sox11 · Expression · Zebrafish · Retina · Optic nerve · Regeneration

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Introduction

In the adult mammalian central nervous system (CNS), most neurons are not replaced following traumatic injury or disease. In contrast, teleost fish such as zebrafish exhibit a remarkable capacity for CNS regeneration [1, 2]. Why lower vertebrates like zebrafish possess such a strong regenerative potential while mammals do not is a very important and fundamental question in neuroscience. The mechanisms underlying CNS repair in zebrafish could therefore be used to design novel therapeutic strategies for improving CNS regeneration in mammals.

One good model to study CNS repair is the zebrafish visual system. As part of the CNS, the visual system is more accessible than other regions, making it an ideal system to study mechanisms of CNS regeneration. Following retinal injury in adult zebrafish, the resident Müller glia rapidly dedifferentiate and proliferate into a cycling population of neurogenic precursors that eventually regenerate new neurons and restore vision [3, 4]. Retinal regeneration in zebrafish is regulated by many extracellular cues such as the Hbegf/Wnt/Fgf/TNFα/TGFβ signaling pathways [5–9], as well as cell-intrinsic transcription factors [10–12]. Zebrafish also mount a robust regenerative response after optic nerve injury that regrows severed axons and restores lost vision [13, 14].

The Sox (Sry-related box) transcription factors are named after their shared motif called the SRY box, which is a high-mobility-group (HMG) DNA binding domain [15]. Based on the sequence similarity and genomic alignment, Sox proteins are subdivided into groups A to J [15]. The mammalian SoxC family has three intronless genes: *Sox4*, *Sox11*, and *Sox12* [15]. Known as transcription activators, the SoxC genes are widely expressed in neuronal progenitors in the brain as well as in other organs during embryogenesis [16, 17]. It has been reported that SoxC members are regulators of cell survival and

proliferation, as well as cell fate and differentiation [16, 18, 19]. In the developing vertebrate eye, SoxC genes exhibit a partially-overlapping expression pattern in the neural retina and lens placode [16, 20, 21]. As retinal neurons mature, SoxC expression is down-regulated and very little expression is detectable in the adult mammalian retina [22]. SoxC genes play multiple roles during vertebrate eye development, including ocular morphogenesis, lens development, and retinal neurogenesis [23]. In mice, *Sox4/Sox11* conditional knockout leads to a significant loss of retinal ganglion cells (RGCs) and other retinal neurons [24]. Interestingly, Sox4- and Sox11-deficient zebrafish exhibit no significant defects in RGC development. Instead, they have reduced number of mature rod photoreceptors in the developing retina [21], suggesting a role of SoxC genes in photoreceptor differentiation.

Although the role of SoxC transcription factors in eye development has been reported previously, their expression and function during retinal and optic nerve regeneration remain largely unknown. Here we report the temporal and spatial expression patterns of SoxC genes during retinal and optic nerve regeneration in zebrafish. We also used morpholino (MO)-based gene knockdown to explore their functions during these regenerative events.

Materials and Methods

Animals and Eye Injury

The zebrafish used in this study were treated in accordance with the guidelines for animal use and care at Nantong University. The *Tg(1016tuba1a:GFP)* transgenic line was generated using the 1016-bp goldfish *tuba1a* promoter as described previously [25]. The retina was injured as described previously [25]. Briefly, fish were anesthetized by immersion in 0.02% Tricaine (Sigma-Aldrich Corp., St. Louis, MO) and the right retina was poked four times, once in each quadrant, with a 30G needle. The needle was inserted through the sclera to the length of the bevel ($\sim$0.5 mm). The optic nerve injury was performed as described previously [13]. Briefly, the right eye was gently pulled out of the socket and the dorsal connective tissue was cut. The optic nerve was cut with iridectomy scissors, taking care not to damage the ophthalmic artery. The eye was then gently replaced in the socket and the fish allowed to recover in system water. The intact left optic nerve served as the control.

RT-PCR and Quantitative PCR (qPCR)

Retinas were isolated and the total RNA was extracted with TRIzol reagent (Thermo Fisher Scientific, Waltham, MA). One microgram of total RNA was reverse-transcribed into cDNA using a Transcriptor First Strand cDNA Synthesis Kit (Roche Applied Science, Penzberg, Upper Bavaria, Germany). The primers for PCR and quantitative PCR (qPCR) are listed in Table 1. qPCR was carried out in triplicate using FastStart Universal SYBR Green Master Mix (Roche Applied Science) on a real-time PCR detection system (CFX96TM Real-Time System, Bio-Rad, Hercules, CA). The relative mRNA expression in intact and injured retinas was determined using the $\Delta\Delta$Ct method and normalized to *gapdh* mRNA expression.

Tissue Preparation and Immunofluorescence

Fish were euthanized with an overdose of Tricaine. The eyes were dissected out and fixed in 4% paraformaldehyde at 4 °C overnight. Fixed samples were cryosectioned and prepared for immunofluorescence as previously described [10]. The primary antibodies for immunofluorescence were rabbit anti-GFP (1:1000, Life Technologies, Carlsbad, CA), and rat anti-BrdU (1:500, Abcam, Cambridge, MA). For BrdU staining, sections were first treated with 2 N HCl at 37 °C for 25 min, rinsed in 0.1 mol/L sodium borate (pH 8.5) for 10 min, and then processed using standard procedures.

BrdU Incorporation and In Situ Hybridization (ISH)

For BrdU incorporation, 20 μL of 20 mmol/L BrdU was injected i.p. into the anesthetized fish 3 h before sacrifice.

Table 1 Primers used in this study.

Gene name	Primer sequence (5′-3′)
gapdh-F	ATGACCCCTCCAGCATGA
gapdh-R	GGCGGTGTAGGCATGAAC
ascl1a-F	GGTGGCCAGACGGAACGAG
ascl1a-R	TTCCCGCTCTTCCCTCTTAGTCC
sox4a-F	TCGCGAGCGGAAAGGAAGA
sox4a-R	CCGCGCAATGCCTTCCTC
sox4b-F	CCAGCGGCCACATCAAGAGA
sox4b-R	GTATCCGCGTCCTCCTTGCC
sox11a-F	GCCACCGGACACATAAAGCG
sox11a-R	GCGCGGAGGAGGCGAAGT
sox11b-F	ACGACGATGATGATGATGACGAC
sox11b-R	CACAGCACAGTCAATGTTTGGACC
sox12-F	ATGCGGGACAGAGGAAGAAAACA
sox12-R	GGGGCTTGCTGGATTTCATAGG
RT-*sox4a*-F	GAGCAGTCGCCGGACATGC
RT-*sox4a*-R	AGTTTTCGAGCTCTTTTTGGATGC
RT-*sox4b*-F	ACACCGGCAGCATGCGC
RT-*sox4b*-R	ACCTCGGGCGTGCAGTAATCG
RT-*sox11a*-F	TCCCAAACTGAAAGCGAACAAGAC
RT-*sox11b*-F	AGCAGCCGCAGGGAAGAAGT
RT-*sox12*-R	TGATGCAGCGCTGAGATCTA

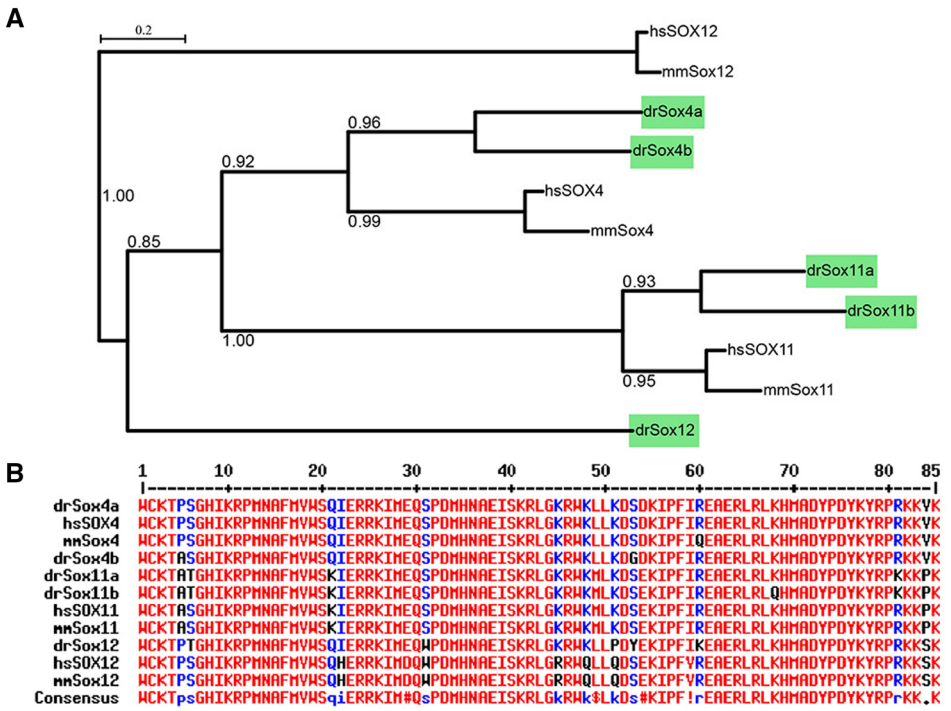

Fig. 1 Zebrafish SoxC genes are homologs of their mammalian counterparts. **A** Phylogenetic tree depicting the evolutionary relationships of human, mouse, and zebrafish SoxC genes generated by PhyML software (http://www.atgc-montpellier.fr/phyml/). Bootstrap values are indicated at each branch point. **B** Multiple alignment of the amino-acid sequences of the HMG domain of SoxC genes. High consensus sequences (>90%) are shown in *red*, and low consensus sequences (>50%) are in *blue*; hs, *Homo sapiens*; mm, *Mus musculus*; dr, *Danio rerio.*

ISH was performed using DIG-labeled cRNA probes on retinal cryosections (DIG-RNA labeling kit, Roche Applied Science) as described previously [10].

Quantification of BrdU+ Cells at the Injury Site

GFP and BrdU immunofluorescence on retinal cryosections showed that each injury-responsive zone (4 injuries per retina) was clearly distinguishable from other areas. The counting area for each injury site was ~400–600 µm in width, based on the actual size. The number of BrdU+ cells per injury in the inner nuclear layer (INL) was counted using the Cell Counter plugin of ImageJ software (Plugin/Analyze/Cell Counter, https://imagej.nih.gov/ij/).

Morpholino Treatments

For retinal injury, 1 µL of 1 mmol/L lissamine-tagged antisense MO (Gene Tools, Philomath, OR) was injected with a Hamilton syringe into the vitreous at the time of injury. Electroporation of MOs was performed as previously described [26]. MO-mediated target gene knockdown in RGCs was performed as previously described [27]. Briefly, gelfoam was cut into small pieces and applied with 1 µL of 1 mmol/L lissamine-tagged MO. The small piece of gelfoam was placed at the cut site of the lesioned optic nerve for 24 h and then removed. A standard control MO was used as the negative control. Translation-blocking MOs targeting zebrafish *sox11a* and *sox11b* are referred to as *sox11a* MO (5′-CCGTTGCCGTGCGTTGTCAGTCCAA-3′) and *sox11b* MO (5′-CATGTTCAAACACACTTTTCCCTCT-3′). Both have been previously described and validated [27].

Retinal Explant Assay

Retinal explant assays were performed as previously described [13, 28]. Briefly, 4 days after optic nerve transection, fish were anesthetized and retinas were isolated. Retinas were cut into 0.5-mm squares, digested with 1 mg/mL hyaluronidase in L15 medium (Life Technology) for 15 min, and then washed 3 times in culture medium (8% fetal calf serum, 3% zebrafish embryo extract, and 1× antibiotic/antimycotic in L15). The 35-mm plates were precoated with 100 µg/mL poly-*L*-lysine and 10 µg/mL laminin overnight. Retinal squares were then plated in the culture medium (one retina per plate) and maintained at 28 °C for 4 days in a humidified air chamber. Axon length and density were measured from images of adherent explants with Leica Qwin software (Leica Camera AG, Wetzlar, Germany). Axon density was defined as the number of neurites per explant. At least three retinas were used for each group and images of 10–15 explants from each retina were captured and quantified.

Microscopy and Statistical Analysis

Bright-field and fluorescence images of retinal cryosections were captured with a Leica DM4000B or a Zeiss Imager

M2 upright microscope (Carl Zeiss AG, Oberkochen, Germany). Images of retinal explants were captured with an Olympus IX51 inverted microscope (Olympus Corporation, Tokyo, Japan). All experiments were repeated at least three times. For single comparisons, a two-tailed unpaired Student's *t*-test was used. For multiple comparisons, one-way analysis of variance (ANOVA) followed by the Tukey test was used.

Results

Expression of SoxC Genes in the Retina After Needle-Poke Injury

By searching the zebrafish genome (http://www.ensembl.org, Zv9), five SoxC members were found: Sox4a, Sox4b, Sox11a, Sox11b, and Sox12. Like mammalian SoxC genes, the zebrafish SoxC members are intronless except for Sox12, which has two exons (data not shown). Zebrafish SoxC members are homologs of their mammalian counterparts, as shown by phylogenetic analysis (Fig. 1A). The amino-acid sequences of the HMG domain in zebrafish and mammalian SoxC proteins have a high similarity (Fig. 1B). These results indicate that the SoxC proteins were highly conserved during vertebrate evolution, suggesting the importance of their functions.

To investigate the temporal expression of zebrafish SoxC genes after retinal injury, retinas were collected at different time points after injury and the total RNA was extracted. RT-PCR results showed that among the five SoxC genes, only *sox11a* and *sox11b* were induced after the injury (Fig. 2A). Specifically, *sox11a* was weakly induced from 14 h post-injury (hpi) to 7 days post-injury (dpi) and declined at 14 dpi (Fig. 2A). The expression of *sox11b* was up-regulated at 14 hpi, reached a peak at 4 dpi, then declined and returned to near the basal level from 7 to 14 dpi (Fig. 2A). Although the transcripts of other SoxC genes were also detected, they showed no evident changes after injury (Fig. 2A). Interestingly, the expression pattern of *sox11b* following injury was similar to that of *ascl1a* (Fig. 2A), which is a key transcription factor regulating retinal regeneration [8, 10]. We next quantified the expression levels of the SoxC genes by qPCR analysis. At 4 dpi, qPCR detected a 2.8-fold increase of *sox11a* and an 8.4-fold increase of *sox11b* mRNA in the retina (Fig. 2B). The fold changes of other SoxC genes were no more than 2 (Fig. 2B). These results indicate that Sox11b is the major SoxC member induced in the retina after needle-poke injury.

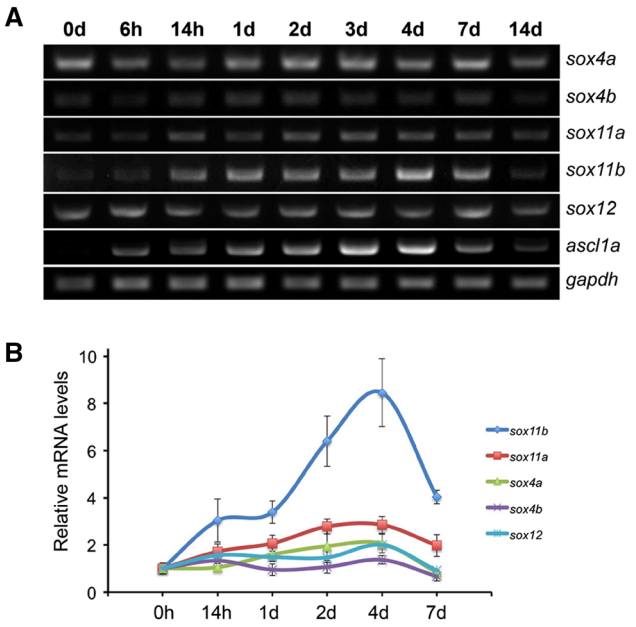

Fig. 2 Temporal expression of zebrafish SoxC genes during retinal regeneration. **A** PCR results of the expression of zebrafish SoxC genes (*sox4a*, *sox4b*, *sox11a*, *sox11b*, and *sox12*) in the whole retina at the indicated time points. The pro-neural gene *ascl1a* served as a positive control for retinal regeneration, and *gapdh* as a loading control. **B** qPCR analysis of the expression of zebrafish SoxC genes after retinal injury at indicated time points ($n = 3$ biological replicates).

Sox11b is Expressed in BrdU+ Cells in the INL After Needle-Poke Injury

To determine where the SoxC genes are expressed in the intact and injured retina, ISH was carried out on retinal cryosections at 0 and 4 dpi. None of the SoxC genes showed a detectable signal in the uninjured retina (Fig. 3A–E), while very weak signals were found for *sox4a*, *sox4b*, and *sox12* at the injury site at 4 dpi (Fig. 3F, G, J). Interestingly, *sox11a* was only weakly induced in the INL but showed a stronger signal in the ganglion cell layer (GCL) around the injury site at 4 dpi (Fig. 3H), suggesting that this gene plays a more important role during optic nerve regeneration than during retinal regeneration. Consistent with the RT-PCR and qPCR results, *sox11b* displayed a strong *in situ* signal both in the INL and the GCL at the injury site at 4 dpi (Fig. 3I). These results suggest that Sox11b, rather than Sox11a, plays an important role during retinal regeneration in adult zebrafish.

It has been shown that almost all BrdU+ cells in the INL at 4 dpi are derived from Müller glia and are multipotent progenitor cells [25, 29]. BrdU+ cells in the INL at 4 dpi thus represent injury-induced Müller glia-derived progenitor cells (MGPCs). By using the transgenic line *Tg(1016tuba1a:GFP)*, in which GFP is specifically induced in de-differentiated Müller glia and MGPCs after injury [25], we found that *sox11b* mRNA co-localized with BrdU and GFP signals in the INL at 4 dpi (Fig. 3K–N, arrows), suggesting that *sox11b* is likely expressed in proliferating MGPCs after retinal injury.

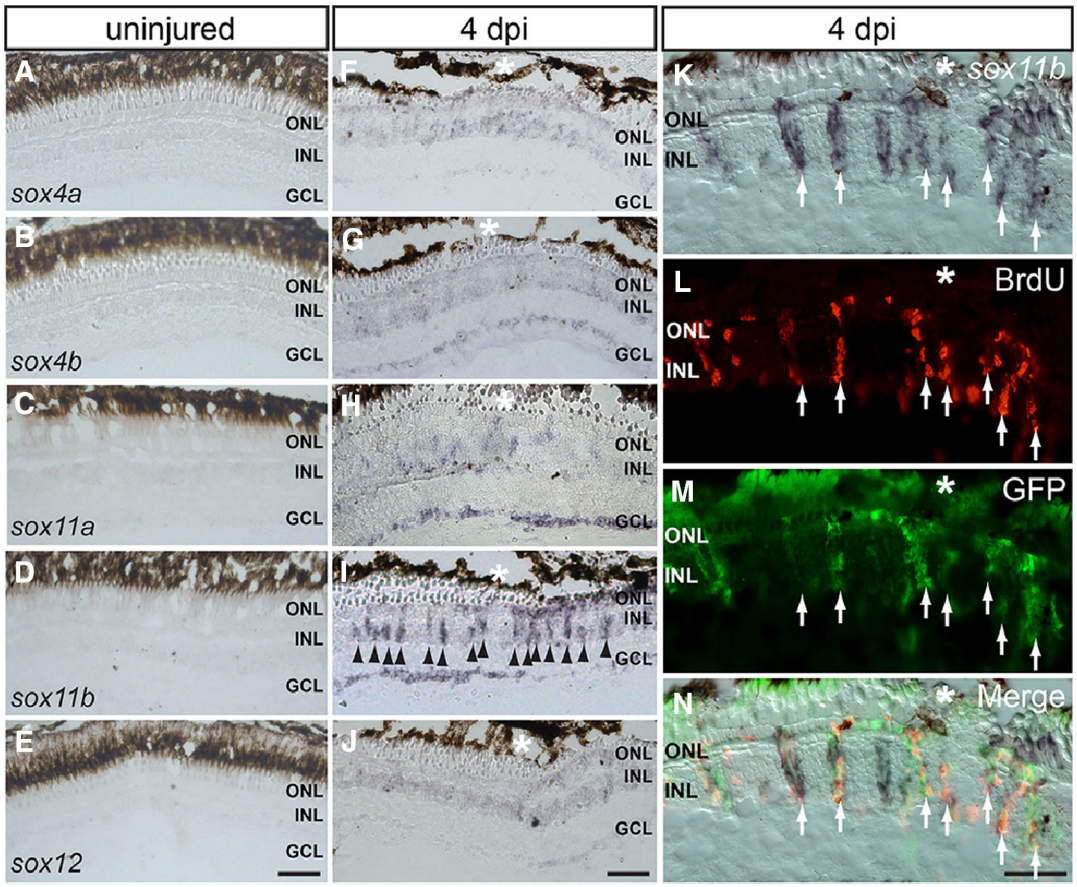

Fig. 3 Spatial expression patterns of SoxC genes in the injured and intact retina. **A–E** *In situ* hybridization (ISH) of all SoxC genes on cryosections of the uninjured retina. **F–J** ISH showing the expression of SoxC genes in the injured retina at 4 dpi. **K–N** ISH of *sox11b* combined with BrdU/GFP immunofluorescence showing its expression in BrdU+/GFP+ cells in the INL at 4 dpi (arrows, co-localization of *in situ*, GFP and BrdU signals; *injury site; *ONL* outer nuclear layer, *INL* inner nuclear layer, *GCL* ganglion cell layer; *scale bars* 50 μm).

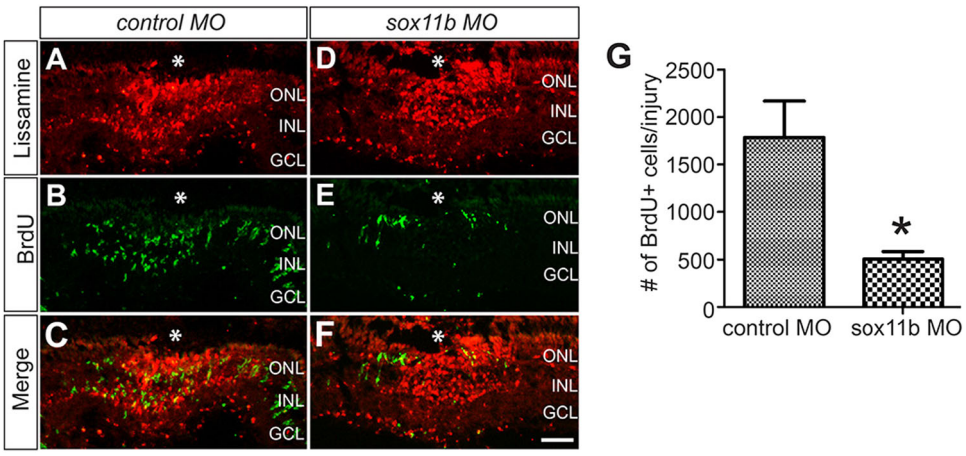

Fig. 4 Sox11b knockdown decreased the number of BrdU+ cells in the INL. **A–F** MO-mediated Sox11b-knockdown reduced the number of BrdU+ cells (*green*) in the INL at 4 dpi. Successful electroporation of lissamine-tagged MOs into the retina was confirmed by red fluorescence at the injury site (*injury site; *scale bar* 50 μm). **G** Quantification of the number of BrdU+ cells in the INL at the injury site ($n = 9$ biological replicates; $^*P < 0.05$; *MO* morpholino, *ONL* outer nuclear layer, *INL* inner nuclear layer, *GCL* ganglion cell layer).

Sox11b Knockdown Decreases the Number of BrdU+ Cells in the INL at 4 dpi

To investigate whether Sox11b is necessary for retinal regeneration, we knocked it down by electroporation of lissamine-tagged control or *sox11b* antisense MOs into the retina at the time of injury. It is important to note that the efficacy of the *sox11a* and *sox11b* MOs used in this study has been described and validated in a previous paper [27]. Cryosections of the electroporated retina showed strong red

fluorescence at the injury site at 4 dpi (Fig. 4A, D), indicating that the lissamine-tagged control and *sox11b* MOs were efficiently transferred into the retina. Strikingly, knockdown of Sox11b resulted in a significantly reduced number of BrdU+ cells in the INL at the injury site compared to the control at 4 dpi (Fig. 4B–G). This suggests that Sox11b plays an important role during retinal regeneration in adult zebrafish.

Expression of SoxC Genes During Zebrafish Optic Nerve Regeneration

The induction of *sox11a* and *sox11b* in the GCL after a needle-poke injury that damaged all the retinal layers in a small region (Fig. 2) suggested that SoxC genes may be induced in the regenerating retinal ganglion cells after optic nerve transection. We therefore performed PCR and qPCR to examine the temporal expression of SoxC genes in the uninjured and optic nerve-lesioned retina. Indeed, PCR and qPCR revealed a strong upregulation of *sox11a* and *sox11b* after the injury, whereas other SoxC members showed no induction except for *sox4b*, which displayed a small induction at 4 dpi (Fig. 5A, B). Specifically, *sox11a* and *sox11b* were both upregulated at 1 dpi, showed strong induction during 2–7 dpi, and then returned close to the basal level at 14 dpi (Fig. 5A, B). At 4 dpi, qPCR detected a 7.9-fold increase of *sox11a* and a 9.1-fold increase of *sox11b* (Fig. 5B). ISH results showed that at 4 dpi, *sox4a* and *sox4b* were weakly expressed in the GCL (Fig. 6A, B), and *sox12* was undetectable (Fig. 6E). In contrast, *sox11a* and *sox11b* were strongly induced in the GCL (Fig. 6C, D), suggesting that they play roles in optic nerve regeneration.

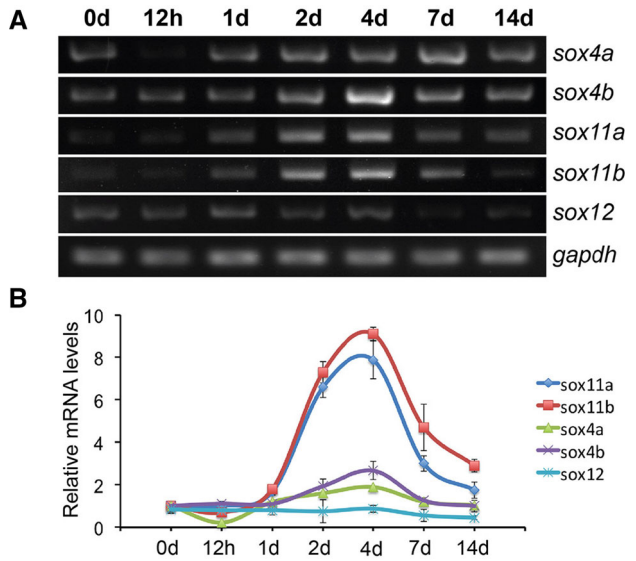

Fig. 5 Temporal expression of SoxC genes after optic nerve transection. **A** PCR results of the expression of zebrafish SoxC genes (*sox4a*, *sox4b*, *sox11a*, *sox11b*, and *sox12*) in the whole retina at indicated time points (*gapdh* was used as a loading control). **B** qPCR analysis of the expression of zebrafish SoxC genes after optic nerve injury at indicated time points (n = 3 biological replicates).

Sox11a and Sox11b are Required for Optic Axon Regeneration

To investigate whether Sox11a and Sox11b are required for optic nerve regeneration in adult zebrafish, we combined *in vivo* MO-mediated protein knockdown in RGCs with retina explants to assay RGC axon regeneration [13]. Retinas were isolated 4 days after optic nerve lesion and MO treatment, diced, and cultured as explants for 4 days. RGC axon outgrowth was then quantified as previously described [28]. Our results showed that explants prepared from control MO-treated retinas displayed robust axon outgrowth (Fig. 7A). Separate knockdown of Sox11a or Sox11b had no significant effect on optic axon regrowth compared to control (Fig. 7A–C, E, F). Sox11a+11b knockdown slightly reduced the average length of regenerated axons but the difference was not significant (Fig. 7A, D, E). However, the average number of axons per explant from Sox11a+11b MO-treated retinas was significantly lower than that of control (Fig. 7A, D, F). The results suggest that Sox11a and Sox11b play important roles during optic nerve regeneration *in vivo*.

Discussion

SoxC transcription factors play important roles during vertebrate ocular morphogenesis and retinal development. However, their expression and function in retinal and optic nerve regeneration remain elusive. In this report, we showed that among the five SoxC genes, only *sox11b* was strongly induced in BrdU+ cells in the INL after retinal injury, and was required for retinal regeneration in zebrafish. During optic nerve regeneration, both *sox11a* and *sox11b* were upregulated in RGCs and were required for optic axon regrowth. Our study thus uncovered the expression pattern of SoxC genes and suggested their important roles during zebrafish retinal and optic nerve regeneration.

During ocular and retinal development, Sox4 and Sox11 showed partially-overlapping expression patterns and redundant functions [23]. In mice, Sox4 and Sox11 are both widely expressed in the neuroblast layer and GCL of the developing retina and share a similar expression pattern [24]. During zebrafish embryonic development, Sox4 (Sox4a/4b) and Sox11 (Sox11a/11b) are also strongly expressed in the developing retina from 24 to 72 h post-fertilization [21, 30]. Because tissue regeneration following injury or disease is often similar to embryonic

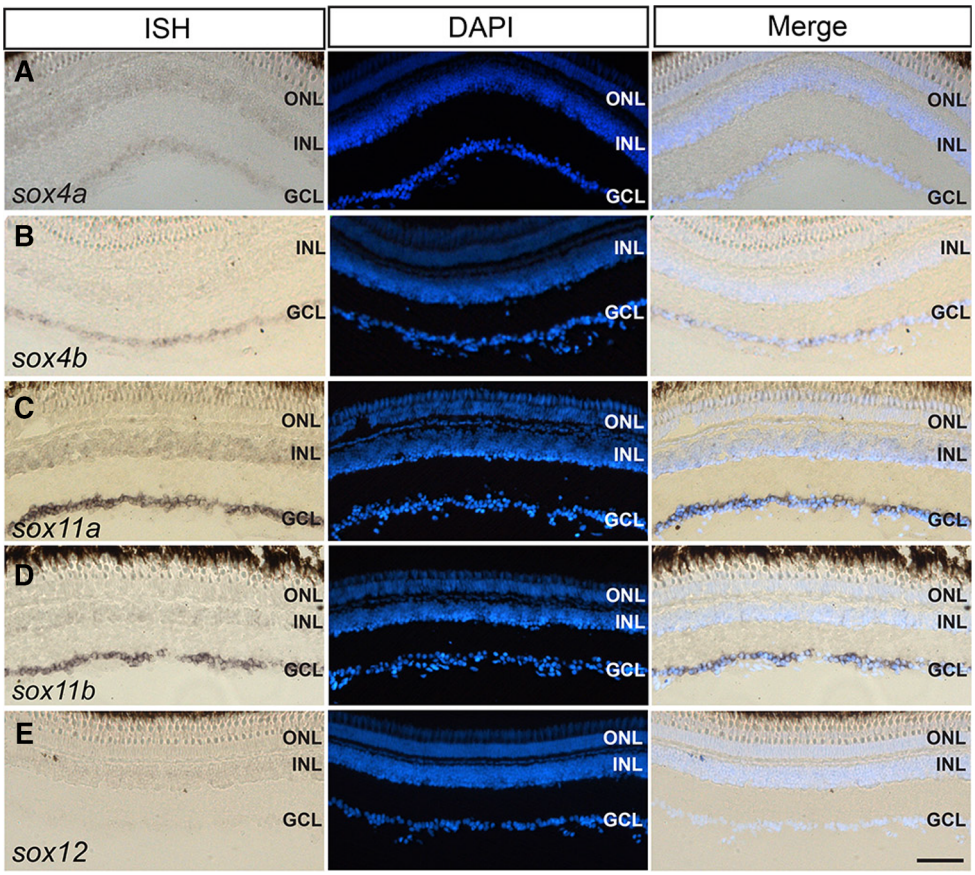

Fig. 6 ISH of zebrafish SoxC genes combined with DAPI staining on cryosections of the injured retina at 4 days after optic nerve injury. Weak *in situ* signals of *sox4a* (**A**) and *sox4b* (**B**) were detected in the GCL. Strong *in situ* signals of *sox11a* (**C**) and *sox11b* (**D**) were detected in the GCL, and no *sox12* signal was detectable (**E**) (*scale bar* 50 μm).

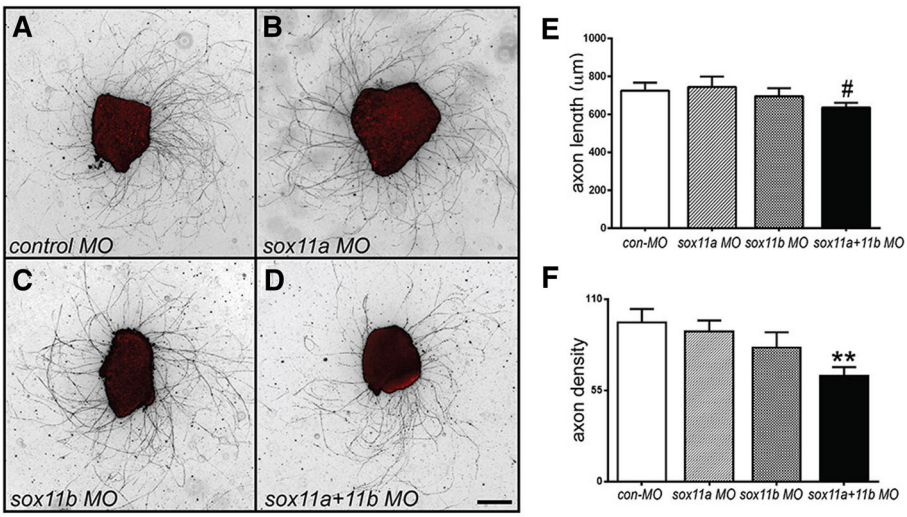

Fig. 7 MO-mediated Sox11a+11b knockdown suppressed optic axon regrowth in explants. **A–D** Representative images of retinal explants showing the outgrowth of regenerated RGC axons from control (**A**) and Sox11a/11b MO-treated retinas (**B–D**). Retinas were isolated 4 days after optic nerve transection, diced, and cultured for another 4 days before analysis of neurite outgrowth. The red fluorescence is from lissamine-tagged control or Sox11a/11b MOs (*scale bar*, 200 μm). **E**, **F** Quantification of axon length and density (mean ± SD, $n = 6$ individual fish; con-MO, control MO; $^{**}P < 0.01$ compared to control).

development, we hypothesized that both Sox4 and Sox11 would be strongly expressed in the regenerating retina. Surprisingly, our results demonstrated that only Sox11 (Sox11a/11b) was strongly induced during retinal and optic nerve regeneration. In contrast, Sox4 (Sox4a/4b) and Sox12 were either not expressed or expressed very weakly

after retinal and optic nerve injury. Why Sox11 is the only SoxC gene induced during regeneration is still unknown. As Sox4 and Sox11 are functionally redundant, it is possible that expression of Sox11 itself is sufficient to regenerate a damaged retina. Our finding is therefore a good example showing that although development and regeneration are similar, they still differ in many ways.

Sox11 plays important roles during ocular morphogenesis and retinal development. In zebrafish, MO-mediated Sox11 knockdown results in delayed and abnormal lens formation, coloboma, and reduced rod photoreceptors due to elevated Hedgehog signaling [21]. In mice, *Sox11* conditional knock-out (KO) leads to a moderate reduction of RGC generation in the retina [24]. In another study, *Sox11* KO mice showed delayed neurogenesis and differentiation of RGCs and cone photoreceptors [31]. These studies demonstrate that Sox11 is an important transcription factor regulating retinal neurogenesis during embryonic development. Here, we showed that after retinal injury, Sox11b was rapidly induced in the retina and was expressed in BrdU+/GFP+ cells (presumably MGPCs) in the INL (Figs. 2 and 3). In addition, MO-mediated Sox11b knockdown significantly decreased the number of proliferating cells in the INL at the injury site, suggesting that Sox11b is essential for MGPC formation and retinal regeneration. The molecular mechanism underlying the function of Sox11b in retinal regeneration is currently unknown and requires further investigation. Since Sox11 regulates cell proliferation [32], it is possible that Sox11b promotes the proliferation of Müller glia and MGPCs during retinal regeneration. It is also possible that Sox11b is required in early retinal regeneration, such as Müller glia reprogramming and de-differentiation.

Sox11 is widely expressed in the developing CNS and peripheral nervous system (PNS) neurons during periods of axon growth and is then down-regulated in the adult stage [32]. In response to injury, Sox11 is rapidly induced in peripheral neurons and promotes axon regeneration [33–36]. However, Sox11 induction is missing after injury in the mammalian CNS where regeneration does not occur [37, 38]. Recently, it was reported that forced expression of Sox11 in mouse corticospinal tract (CST) neurons promotes CST regeneration after spinal injury [38]. Therefore, Sox11 may function as a key transcription factor that enhances the intrinsic regenerative capacity of CNS neurons. Consistently, we showed that Sox11a and Sox11b were strongly induced in RGCs following optic nerve transection in adult zebrafish, and were required for optic axon regrowth in a retinal explant assay. The phenotype of axon regrowth after Sox11a/11b knockdown was moderate and this may be due to the presence of other SoxC genes in RGCs such as Sox4a and Sox4b. Our result differs from a previous report showing that Sox11a/11b are not required for RGC axon regrowth in adult zebrafish [27]. We speculate that the difference might be due to the different fish lines used, the conditions for retinal explants, and the amounts of MOs retained in the RGCs after gelfoam treatment. Further *in vivo* experiments, particularly functional studies, are required to clarify the roles of Sox11a/11b in RGCs after optic nerve injury.

Acknowledgements This work was supported by grants from National Natural Science Foundation of China (31401234), Natural Science Foundation of Jiangsu Province, China (BK20140428), the Basic Research Program of Education Department of Jiangsu Province, China (14KJB180019), and the Science and Technology Project of Nantong Municipality, Jiangsu Province, China (MS22015002).

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